# Glutarimide Alkaloids and a Terpenoid Benzoquinone from *Cordia globifera*

Joshua Parks,[†,‡] Thinley Gyeltshen,[§] Vilailak Prachyawarakorn,[†] Chulabhorn Mahidol,[†,§,⊥] Somsak Ruchirawat,[†,§,⊥] and Prasat Kittakoop*[,†,§]

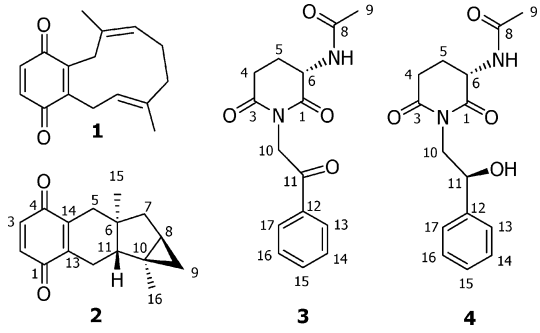
*Chulabhorn Research Institute, Vibhavadi-Rangsit Road, Laksi, Bangkok 10210, Thailand, Department of Chemistry, University of Portland, 5000 N. Willamette Boulevard, Portland, Oregon 97203, Chemical Biology Program, Chulabhorn Graduate Institute, and the Center for Environmental Health, Toxicology and Management of Chemicals (ETM), Vibhavadi-Rangsit Road, Laksi, Bangkok 10210, Thailand, and Chulabhorn Research Centre, Institute of Molecular Biosciences, Mahidol University, Thailand*

*Received February 2, 2010*

Three new compounds, a meroterpene (**2**) having a cyclopropane moiety named globiferane and glutarimide alkaloids named cordiarimides A (**3**) and B (**4**), were isolated from the roots of *Cordia globifera*. Compounds **2**−**4** exhibited weak cytotoxic activity. Cordiarimide B (**4**) exhibited radical scavenging activity, as it inhibited superoxide anion radical formation in the xanthine/xanthine oxidase (XXO) assay, and also suppressed superoxide anion generation in differentiated HL-60 human promyelocytic leukemia cells when induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). This is the first report on the presence of glutarimide alkaloids in the genus *Cordia*.

The genus *Cordia,* family Boraginaceae, is native to tropical America, Africa, and Asia, and compounds isolated from this genus have displayed a broad range of biological activities, including antiandrogenic,[1] anti-inflammatory,[2] antifungal,[3,4] and larvacidal properties.[4] *Cordia globifera* W. W. Smith (Boraginaceae) is locally known in Thai as "Sak Hin". We previously reported the isolation of globiferin (**1**) and some of its derivatives from *C. globifera*, as well as the biomimetic transformation and antimalarial, antimycobacterial, antifungal, and cytotoxic activities of these substances.[5] Because **1** exhibited significant antimycobacterial activity, with the MIC value of 6.2 $\mu$g/mL,[5] we re-collected *C. globifera* for reisolation of **1** and for further detailed studies toward multi-drug-resistant strains of *Mycobacterium tuberculosis*. In addition to **1**, we found a new meroterpene displaying a cyclopropane moiety (**2**) and new glutarimide alkaloids (**3** and **4**) from a root extract of *C. globifera*. To our knowledge, this is the first report on the presence of glutarimide alkaloids in the genus *Cordia*.

Extraction of *C. globifera* roots yielded globiferin (**1**) as described in our previous report.[5] The MeOH-soluble part of the $CH_2Cl_2$ extract of *C. globifera* roots, separated using Sephadex LH-20 and silica gel chromatographic techniques (see Experimental Section), also yielded previously unknown compounds **2**−**4**.



Compound **2** had the molecular formula $C_{16}H_{18}O_2$, as revealed by the ESITOF-MS spectrum. The $^1$H and $^{13}$C NMR spectra revealed the presence of a 1,4-benzoquinone unit in **2**, showing signals at $\delta_H$ 6.70 (1H, d, $J$ = 11.9 Hz, H-2) and 6.72 (1H, d, $J$ = 11.9 Hz, H-3) and at $\delta_C$ 187.7 (C-4) and 187.4 (C-1), which were similar to those of **1**.[5] Upfield $^1$H NNR resonances ($\delta_H$ 0.35 and 0.94; $H_2$-9) of a nonequivalent methylene suggested that **2** possessed a cyclopropane moiety. The presence of two methyl, four methylene, four methine, and six nonprotonated carbons in **2** was revealed by $^{13}$C and DEPT NMR spectra. The $^1$H−$^1$H COSY spectrum of **2** established the fragment $H_2$-7/H-8/$H_2$-9 and also showed couplings between H-2 and H-3 and between H-11 and H-12. Allylic coupling between $H_2$-5 and $H_2$-12 was also observed in the $^1$H−$^1$H COSY spectrum of **2**. The gross structure of **2** was established by the following HMBC correlations: H-2 to C-4 and C-13; H-3 to C-1 and C-14; $H_2$-5 to C-6, C-7, C-11, and C-15; H-8 to C-11; $H_2$-7 to C-9, C-10, C-11, and C-15; $H_2$-9 to C-7, C-10, and C-11; H-11 to C-5, C-6, C-9, C-10, C-13, and C-15; $H_2$-12 to C-1, C-6, and C-14; $H_3$-15 to C-5, C-6, C-7, and C-11; and $H_3$-16 to C-8, C-9, C-10, and C-11. The relative configuration of **2** was assigned by analysis of the NOESY spectrum. No NOESY cross-peak was observed between H-11 and $H_3$-15, indicating the *trans*-fused ring junction at C-6/C-11. The cyclopropane moiety was coplanar with H-11 because there was an intense NOESY cross-peak between H-9$\beta$ ($\delta_H$ 0.35) and H-11 ($\delta_H$ 1.15). The NOESY correlations among H-9$\beta$ ($\delta_H$ 0.35), H-7$\beta$ ($\delta_H$ 1.01), and H-5$\beta$ ($\delta_H$ 2.10) suggested that they were in the same ($\beta$) plane. The NOESY correlations also indicated that $H_3$-15 ($\delta_H$ 0.86), H-5$\alpha$ ($\delta_H$ 2.55), H-7$\alpha$ ($\delta_H$ 1.99), and H-12$\alpha$ ($\delta_H$ 2.25) were coplanar; there were correlations among these protons. It is worth noting that the six- and five-membered-ring fused system of **2** is *trans*, while the six- and six-membered-ring junction of this class of compounds (known as terpenoid benzoquinones, i.e., cordiachromes and cordiaquinols) is *cis*.[6−8] Normally terpenoid benzoquinones from *Cordia* species have a 6,6-membered ring junction and are optically inactive.[5−8] Interestingly, **2**, with a 6,5-membered ring junction and a cyclopropane unit, is optically active ($[\alpha]^{26}_D$ −96). Compound **2**, which was named globiferane, is a derivative of 4,5-dimethoxy-1a,7a-dimethyl-1,1a,1b,2,7,7a,8,8a-octahydrocyclopropa[3,4]cyclopenta[1,2-*b*]naphthalene-3,6-dione, a meroterpenoid benzoquinone previously isolated from *Cordia globosa*.[9] The optical rotation of globiferane (**2**) was different from that of 4,5-dimethoxy-1a,7a-dimethyl-1,1a,1b,2,7,7a,8,8a-octahydrocyclopropa[3,4]cyclopenta[1,2-*b*]naphthalene-3,6-dione ($[\alpha]^{20}_D$ +4.8);[9] however, both compounds share the same relative configuration.

Compound **3** possessed the molecular formula $C_{15}H_{16}N_2O_4$ (deduced from the ESITOF-MS spectrum). The $^1$H NMR spectrum of **3** showed signals of a 1-substituted benzene ring ($\delta_H$ 7.97 (2H, d, $J$ = 7.5 Hz, H-13/H-17), 7.63 (1H, t, $J$ = 7.5 Hz, H-15), and

* To whom correspondence should be addressed. Tel: +66-86-9755777. Fax: +662-5740622, ext. 1513. E-mail: prasatkittakoop@yahoo.com.
† Chulabhorn Research Institute.
‡ University of Portland.
§ Chulabhorn Graduate Institute.
⊥ Mahidol University.

10.1021/np100078s 
Published on Web 04/12/2010

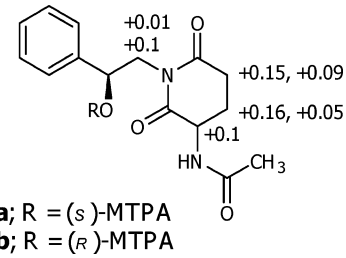



**Figure 1.** $\Delta\delta$ values [$\delta_{(S)} - \delta_{(R)}$] for the MTPA esters **4a** and **4b**.

7.51 (2H, t, $J = 7.5$ Hz, H-14/H-16)); a methine ($\delta_H$ 4.73); three nonequivalent methylenes ($\delta_H$ 1.98−5.27); a methyl group ($\delta_H$ 2.06); and an exchangeable proton ($\delta_H$ 6.44). The DEPT and HSQC spectra revealed that **3** contained a methyl, six methine, three methylene, and five quaternary carbons. $^1$H and $^{13}$C resonances ($\delta_H$ 4.73; $\delta_C$ 51.4) of a H-6 methine, together with the coupling between this methine and an exchangeable proton ($\delta_H$ 6.44, N*H*) observed in the $^1$H−$^1$H COSY spectrum, implied that the C-6 methine was attached to a nitrogen atom, possibly through an amide linkage. The $^1$H−$^1$H COSY spectrum of **3** established the fragment N*H*/H-6/H$_2$-5/H$_2$-4. HMBC correlations from H$_2$-4 to C-3; H$_2$-5 to C-1 and C-3; H-6 to C-1 and C-8; N*H* to C-8; and H$_3$-9 to C-8 established the *N*-acetylglutamate structure in **3**. Additional HMBC correlations from H$_2$-10 to C-1, C-3, C-11, and C-12 and from H-13/H17 to C-11 revealed the structure of **3** as shown, and it was named cordiarimide A. The coupling value ($J_{H\text{-}5,H\text{-}6} = 13.0$ Hz) suggested that H-6 was pseudoaxial. Cordiarimide A (**3**) exhibited a negative optical rotation similar to those of crotonimides A and B[10] and julocrotone,[11] suggesting that its C-6 configuration was *S*. These glutarimides are derivatives of julocrotine ([$\alpha$]$_D$ −9), a glutarimide alkaloid containing an L-glutamic residue in its molecule, which was first isolated from *Julocroton montevidensis*[12] and recently reisolated from *Croton membranaceus*.[13]

Compound **4** was a derivative of **3**, exhibiting the molecular formula $C_{15}H_{18}N_2O_4$ (by ESITOF-MS). The $^1$H NMR spectrum of **4** was similar to that of **3**, except for an additional oxygenated methine ($\delta_H$ 4.90, H-11) in **4**. Analysis of the $^{13}$C NMR spectrum revealed that the ketone resonance ($\delta_C$ 191.4) in **3** was replaced by an oxygenated methine resonance ($\delta_C$ 72.1) in **4**. Thus, **4** had an OH at C-11 rather than a ketone as in **3**. Key HMBC correlations for cordiarimide B (**4**) were observed from H-4 to C-3; H-5 to C-1; H-6 to C-1 and C-8; H-9 to C-8; H-10 to C-1, C-3, C-11, and C-12; H-11 to C-10, C-12, and C-13/C-17; and H-13/17 to C-11 and C-12. The coupling value ($J_{H\text{-}5,H\text{-}6} = 12.8$ Hz) again indicated pseudoaxial orientation of H-6 in **4**. The absolute configuration of the chiral C-11 secondary alcohol in **4** was addressed by a modified Mosher's method;[14,15] both (*S*)- and (*R*)-MTPA esters **4a** and **4b** were separately prepared and subjected to $^1$H NMR analysis. Although $\Delta\delta$ values of the aromatic region could not be calculated due to overlapping signals of the ester **4a** (and **4b**) with those of MTPA, the available $\Delta\delta$ values (Figure 1) implied that the absolute configuration at C-11 of **4**, named cordiarimide B, was likely to be *S*.

Globiferane (**2**) was weakly cytotoxic toward HepG2 (human hepatocellular liver carcinoma), MOLT-3 (acute lymphoblastic leukemia), A549 (human lung carcinoma), and HuCCA-1 (human lung cholangiocarcinoma) cancer cell lines, with respective IC$_{50}$ values of 148.6, 3.7, 148.6, and 66.0 $\mu$M. It should be noted that 4,5-dimethoxy-1a,7a-dimethyl-1,1a,1b,2,7,7a,8,8a-octahydrocyclopropa[3,4]cyclopenta[1,2-*b*]naphthalene-3,6-dione, a derivative of **2**, was reported to display significant cytotoxic activity against several cancer cell lines through DNA synthesis inhibition.[9] Cordiarimides A (**3**) and B (**4**) showed weak cytotoxicity against the MOLT-3 cell line, with IC$_{50}$ values of 145.3 and 44.5 $\mu$M, respectively, and were inactive toward HepG2, A549, and HuCCA-1 cell lines. Cordiarimides A (**3**) and B (**4**) inhibited superoxide anion radical formation in the xanthine/xanthine oxidase (XXO) assay, with respective IC$_{50}$ values of 54.1 and 21.7 $\mu$M. Cordiarimide B (**4**) suppressed superoxide anion generation (54% at 100 $\mu$M) in differentiated HL-60 human promyelocytic leukemia cells when induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). These radical scavenging activities suggest that both **3** and **4** may have potential for cancer chemoprevention.

Glutarimide alkaloids isolated from *Croton cuneatus* were previously reported to exhibit cytotoxic activity.[11] Glutarimide alkaloids have been found in the genus *Croton*,[10,11,13,16,17] except for julocrotine from *Julocroton montevidensis*.[12] This is the first report on the presence of glutarimide alkaloids in the genus *Cordia*. It should be noted that plants of the genus *Cordia* are dioecious, having separate male and female plants. The root sample from our previous study was from a female plant, while the sample reported here was from a male plant, suggesting that glutarimide alkaloids may be present only in the male plant of *C. globifera*.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured at the sodium D line (590 nm) on a JASCO DIP-370 digital polarimeter. UV−vis spectra were obtained using a Shimadzu UV-1700 PharmaSpec spectrophotometer. FTIR data were obtained using a universal attenuated total reflectance (UATR) attachment on a Perkin-Elmer Spectrum One spectrometer. $^1$H, $^{13}$C, and 2-D NMR spectra were recorded on a Bruker AVANCE 600 NMR spectrometer (operating at 600 MHz for $^1$H and 150 MHz for $^{13}$C). ESITOF-MS were determined using a Bruker MicroTOF$_{LC}$ spectrometer.

**Plant Material.** Roots of *C. globifera* were collected from Nakhon Sawan Province, Thailand, in Feburary 2008. Authentication of *C. globifera* was carried out in our previous study,[5] and its voucher specimen (no. CRI 566) was deposited at the Laboratory of Natural Products, Chulabhorn Research Institute.

**Extraction and Isolation.** Roots of *C. globifera* (0.7 kg) were chopped, dried in the shade, ground, and soaked in $CH_2Cl_2$ (three days) at room temperature. The $CH_2Cl_2$ extract was evaporated to yield a sticky residue (3 g), which was partitioned between hexane and MeOH, yielding a hexane-soluble extract (1.5 g) and a MeOH-soluble extract (1.4 g). The hexane-soluble extract was purified following our method described previously,[5] to yield globiferin (**1**, 70 mg). The MeOH-soluble extract was separated by Sephadex LH-20 column chromatography (CC), affording 21 fractions. Combination of similar fractions was guided by TLC to provide seven subfractions (F1−F7). Fraction F4 was separated by silica gel CC, sequentially eluted with a mixture of $CH_2Cl_2$/hexane (1:1) and $Me_2CO/CH_2Cl_2$, yielding 3.4 mg of **2**. Fraction F2 was subjected to Sephadex LH-20 CC affording four fractions (F21−F24). Fraction F23 was further separated by Sephadex LH-20 CC to give 12 fractions (F231−F2312). Fraction F235 was glutarimide **4** (64.3 mg). Fractions F236−F239 were combined and separated by Sephadex LH-20 CC, followed by preparative TLC (developed with a mixture of $Me_2CO/CH_2Cl_2$, 1:3) to afford 10.3 mg of glutarimide **3**.

**Globiferane (2):** yellow oil; [$\alpha$]$_D^{26}$ −96 (*c* 0.11, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 205 (2.83), 290 (sh), 338 (1.44) nm; IR (UATR) $\nu_{max}$ 2923, 2852, 1740, 1653, 1462, 1376, 1271, 1160 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 600 MHz) $\delta$ 6.70 (1H, d, $J = 11.9$ Hz, H-2), 6.72 (1H, d, $J = 11.9$ Hz, H-3), 2.81 (1H, ddd, $J = 2.5, 5.2, 19.1$ Hz, H-12$\beta$), 2.55 (1H, dd, $J = 2.2, 18.9$ Hz, H-5$\alpha$), 2.25 (1H, m, H-12$\alpha$), 2.10 (1H, ddd, $J = 2.5, 2.8, 18.9$ Hz, H-5$\beta$), 1.99 (1H, dd, $J = 6.8, 12.6$ Hz, H-7$\alpha$), 1.24 (1H, m, H-8), 1.22 (3H, s, H-16), 1.15 (1H, dd, $J = 5.2, 13.1$ Hz, H-11), 1.01 (1H, dd, $J = 3.4, 12.6$ Hz, H-7$\beta$), 0.94 (1H, dd, $J = 4.0, 8.3$ Hz, H-9$\alpha$), 0.86 (3H, s, H-15), 0.35 (1H, t, $J = 4.0$ Hz, H-9$\beta$); $^{13}$C NMR ($CDCl_3$, 150 MHz) $\delta$ 187.7 (C=O, C-4), 187.4 (C=O, C-1), 142.7 (C, C-13), 142.6 (C, C-14), 136.5 (CH, C-3), 136.3 (CH, C-2), 50.2 (CH, C-11), 49.1 (C, C-6), 44.8 ($CH_2$, C-7), 36.4 ($CH_2$, C-5), 33.4 ($CH_2$, C-9), 26.6 (CH, C-8), 26.4 (C, C-10), 22.7 ($CH_2$, C-12), 19.6 ($CH_3$, C-15), 19.1 ($CH_3$, C-16); ESITOF-MS *m*/*z* 243.1379 [M + H]$^+$ (calcd for $C_{16}H_{19}O_2$, 243.1385).

**Cordiarimide A (3):** light brown, amorphous solid; [$\alpha$]$_D^{26}$ −29 (*c* 0.36, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 202 (2.15), 242 (1.84) nm; IR (UATR) $\nu_{max}$ 2925, 1735, 1681, 1535, 1404, 1368, 1332, 1227, 1165, 1015, 992, 752, 734, 689 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 600 MHz) $\delta$ 7.97 (2H, d, $J = 7.5$ Hz, H-13/H-17), 7.63 (1H, t, $J = 7.5$ Hz, H-15), 7.51

(2H, t, $J$ = 7.5 Hz, H-14/H-16), 6.44 (1H, d, $J$ = 5.3 Hz, N*H*), 5.27 (1H, d, $J$ = 17.0 Hz, H-10a), 5.18 (1H, d, $J$ = 17.0 Hz, H-10b), 4.73 (1H, ddd, $J$ = 5.4, 5.4, 13.0 Hz, H-6), 2.95 (1H, ddd, $J$ = 2.5, 4.7, 18.0 Hz, H-4eq), 2.86 (1H, ddd, $J$ = 5.3, 13.4, 18.0 Hz, H-4ax), 2.59 (1H, m, H-5ax), 2.06 (3H, s, H-9), 1.98 (1H, ddd, $J$ = 4.7, 13.0, 26.2 Hz, H-5eq); $^{13}$C NMR ($CDCl_3$, 150 MHz) $\delta$ 191.4 (C=O, C-11), 171.8 (C, C-8), 170.9 (C, C-3), 170.3 (C, C-1), 134.6 (C, C-12), 133.9 (CH, C-15), 128.9 (CH, C-14/C-16), 128.0 (CH, C-13/C-17), 51.4 (CH, C-6), 46.4 ($CH_2$, C-10), 31.5 ($CH_2$, C-4), 24.4 ($CH_2$, C-5), 23.1 ($CH_3$, C-9); ESITOF-MS $m/z$ 311.0997 $[M + Na]^+$ (calcd for $C_{15}H_{16}N_2O_4Na$, 311.1008).

**Cordiarimide B (4):** light brown, amorphous solid; $[\alpha]_D^{26}$ +20 ($c$ 0.53, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 209 (1.97), 257 (sh) nm; IR (UATR) $\nu_{max}$ 3344, 2924, 1730, 1658, 1535, 1424, 1374, 1330, 1163, 1096, 1060, 1027, 756, 734, 701 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 600 MHz) $\delta$ 7.39 (2H, d, $J$ = 7.5 Hz, H-13/H-17), 7.36 (2H, t, $J$ = 7.5 Hz, H-14/H-16), 7.29 (1H, t, $J$ = 7.5 Hz, H-15), 6.67 (1H, br d, $J$ = 3.0 Hz, N*H*), 4.90 (1H, dd, $J$ = 2.8, 9.5 Hz, H-11), 4.59 (1H, ddd, $J$ = 6.3, 6.3, 12.8 Hz, H-6), 4.14 (1H, dd, $J$ = 9.6, 13.7 Hz, H-10a), 3.94−3.99 (1H, m, H-10b), 2.84 (1H, dd, $J$ = 2.1, 18.1 Hz, H-4eq), 2.76 (1H, ddd, $J$ = 5.4, 13.5, 18.5 Hz, H-4ax), 2.39−2.44 (1H, m, H-5ax), 2.05 (3H, s, H-9), 1.81−1.89 (1H, m, H-5eq); $^{13}$C NMR ($CDCl_3$, 150 MHz) $\delta$ 172.8 (C, C-1), 172.0 (C, C-3), 170.7 (C, C-8), 141.3 (C, C-12), 128.5 (CH, C-14/C-16), 128.0 (CH, C-15), 125.8 (CH, C-13/C-17), 72.1 (CH, C-11), 51.3 (CH, C-6), 47.6 ($CH_2$, C-10), 31.6 ($CH_2$, C-4), 24.0 ($CH_2$, C-5), 23.0 ($CH_3$, C-9); ESITOF-MS $m/z$ 313.1159 $[M + Na]^+$ (calcd for $C_{15}H_{18}N_2O_4Na$, 313.1164).

**Preparation of (*R*)- and (*S*)-MTPA Esters of Cordiarimide B (4).** To a dry $CH_2Cl_2$ solution (0.5 mL) of **4** (6.9 mg) and *N*,*N*-(dimethylamino)pyridine (5.5 mg) were added dry triethylamine (0.5 mL) and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPACl) (10 $\mu$L). The mixture was stirred at room temperature for 24 h, evaporated, and purified by HPLC ($C_{18}$-Cosmosil MS-II, 10 × 250 mm; eluted with a mixture of MeCN/$H_2O$ (47:53); flow rate 4.0 mL/min) to give (*R*)-MTPA ester (**4b**, 4.6 mg, $t_R$ 21 min). Compound **4** (6.4 mg) was treated with (*R*)-(−)-MTPACl (10 $\mu$L) in the same manner as described above to give (*S*)-MTPA ester (**4a**, 4.0 mg, $t_R$ 23 min). (*S*)-MTPA ester (**4a**): $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 7.42−7.33 (10H, m, aromatic signals of MTPA and **4a**), 6.23 (1H, d, $J$ = 5.6 Hz, N*H*), 6.19 (1H, dd, $J$ = 9.6, 3.9 Hz, H-11), 4.53 (1H, dd, $J$ = 14.0, 9.6 Hz, H-10a), 4.48 (1H, dt, $J$ = 13.0, 5.3 Hz, H-6), 4.00 (1H, dd, $J$ = 14.0, 3.9 Hz, H-10b), 3.51 (3H, s, O*CH*$_3$ of MTPA), 2.83 (1H, ddd, $J$ = 18.0, 4.4, 2.4 Hz, H-4eq), 2.65 (1H, ddd, $J$ = 18.0, 13.6, 5.4 Hz, H-4ax), 2.47 (1H, m, H-5ax), 2.077 (3H, s, NCO*CH*$_3$), 1.65 (1H, m, H-5a). ESITOF-MS (positive ion mode): $m/z$ 529.1562 $[M + Na]^+$ (calcd for $C_{25}H_{25}F_3N_2NaO_6$, 529.1556). (*R*)-MTPA ester (**4b**): $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 7.44−7.32 (10H, m, aromatic signals of MTPA and **4b**), 6.16 (1H, dd, $J$ = 10.0, 3.9 Hz, H-11), 6.15 (1H, N*H*), 4.52 (1H, dd, $J$ = 14.0, 10.0 Hz, H-10a), 4.38 (1H, dt, $J$ = 13.0, 5.3 Hz, H-6), 3.90 (1H, dd, $J$ = 14.0, 3.9 Hz, H-10b), 3.45 (3H, s, O*CH*$_3$ of MTPA), 2.74 (1H, ddd, $J$ = 18.0, 4.4, 2.2 Hz, H-4eq), 2.50 (1H, ddd, $J$ = 18.0, 13.7, 5.4 Hz, H-4ax), 2.42 (1H, m, H-5ax), 2.078 (3H, s, NCO*CH*$_3$), 1.49 (1H, m, H-5eq). ESITOF-MS (positive ion mode): $m/z$ 529.1558 $[M + Na]^+$ (calcd for $C_{25}H_{25}F_3N_2NaO_6$, 529.1556).

**Cytotoxicity Assay.** Cytotoxic activity for HepG2, HuCCA-1, and A549 cancer cell lines was evaluated with the MTT assay,[18] while that for MOLT-3 and HL-60 cell lines was assessed using the XTT assay.[19] Doxorubicin was used as the reference drug, and respective $IC_{50}$ values of 0.37, 0.64, 0.40, and 0.04 $\mu$M were observed for the HepG2, HuCCA-1, A549, and MOLT-3 cell lines.

**Inhibition of Superoxide Anion Radical Formation by Xanthine/Xanthine Oxidase (XXO Assay).** The XXO assay was performed following the method essentially described by Gerhauser et al.[20] Superoxide dismutase was used as a control. Allopurinol, the reference compound, inhibited xanthine oxidase (IXO) with an $IC_{50}$ value of 3.0 $\mu$M. Inhibition of superoxide anion radical formation was measured only when the tested compounds did not inhibit xanthine oxidase.

**Inhibition of 12-*O*-Tetradecanoylphorbol-13-acetate (TPA)-Induced Superoxide Anion Radical Generation in Differentiated HL-60 cells (HL-60 Assay).** TPA-induced superoxide anion radical formation was detected in differentiated HL-60 human promyelocytic leukemia cells by photometric determination of cytochrome *c* reduction, following the method previously described by Gerhauser et al.[20] Superoxide dismutase was used as a positive control. Only the test samples with cell viability more than 50% were considered for the calculation of scavenging potential.

**Acknowledgment.** P.K. is grateful to the Thailand Research Fund (grant no. DBG5180014) and the Center for Environmental Health, Toxicology and Management of Chemicals (ETM) for financial support. T.G. thanks the Thailand International Development Cooperation Agency (TICA) for a student scholarship. We thank S. Sitthimonchai for determining radical scavenging activity, and P. Intachote, S. Sengsai, and B. Saimanee for cytotoxicity testing. The NSF-REU supported J.P. for a three-month research study at CRI, Thailand.

**Supporting Information Available:** $^1$H, $^{13}$C, and 2D NMR spectra of **2**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

## References and Notes

(1) Kuroyanagi, M.; Seki, T.; Hayashi, T.; Nagashima, Y.; Kawahara, N.; Sekita, S.; Satake, M. *Chem. Pharm. Bull.* **2001**, *49*, 954–957.
(2) Kahn, H. P.; Cossy, J. *Tetrahedron Lett.* **1999**, 8113–8114.
(3) Ioset, J. R.; Marston, A.; Gupta, M. P.; Hostettmann, K. *J. Nat. Prod.* **2000**, *63*, 424–426.
(4) Ioset, J. R.; Marston, A.; Gupta, M. P.; Hostettmann, K. *Phytochemistry* **2000**, *53*, 613–617.
(5) Dettrakul, S.; Surerum, S.; Rajviroongit, S.; Kittakoop, P. *J. Nat. Prod.* **2009**, *72*, 861–865.
(6) Moir, M.; Thomson, R. H. *J. Chem. Soc., Perkin Trans. 1* **1973**, 1352–1357.
(7) Manners, G. D.; Jurd, L. *J. Chem. Soc., Perkin Trans. 1* **1977**, 405–410.
(8) Mori, K.; Kawano, M.; Fuchino, H.; Ooi, T.; Satake, M.; Agatsuma, Y.; Kusumi, T.; Sekita, S. *J. Nat. Prod.* **2008**, *71*, 18–21.
(9) Alencar de Menezes, J. E.; Lemos, T. L.; Pessoa, O. D.; Braz-Filho, R.; Montenegro, R. C.; Wilke, D. V.; Costa Lotufo, L. V.; Pessoa, C.; de Moraes, M. O.; Silveira, E. R. *Planta Med.* **2005**, *71*, 54–58.
(10) Barbosa, P. S.; Abreu, A. S.; Batista, E. F.; Guilhon, G. M. S. P.; Müller, A. H.; Arruda, M. S. P.; Santos, L. S.; Arruda, A. C.; Secco, R. S. *Biochem. Syst. Ecol.* **2007**, *35*, 887–890.
(11) Suárez, A. I.; Blanco, Z.; Delle Monache, F.; Compagnone, R. S.; Arvelo, F. *Nat. Prod. Res.* **2004**, *18*, 421–426.
(12) Nakano, T.; Djerassi, C.; Corral, R. A.; Orazi, O. O. *J. Org. Chem.* **1961**, *26*, 1184–1191.
(13) Aboagye, F. A.; Sam, G. H.; Massiot, G.; Lavaud, C. *Fitoterapia* **2000**, *71*, 461–462.
(14) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512–519.
(15) Sullivan, G. R.; Dale, J. A.; Mosher, H. S. *J. Org. Chem.* **1973**, *38*, 2143–2147.
(16) Kutney, J. P.; Klein, F. K.; Eigendorf, G.; McNeill, D.; Stuart, K. L. *Tetrahedron Lett.* **1971**, *12, 1971*, 4973–4975.
(17) Stuart, K. L.; McNeill, D.; Kutney, J. P.; Eigendorf, G.; Klein, P. K. *Tetrahedron* **1973**, *29*, 4071–4075.
(18) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936–942.
(19) Doyle, A.; Griffiths, J. B. In *Mammalian Cell Culture: Essential Techniques*; John Wiley & Sons: Chichester, UK, 1997.
(20) Gerhauser, C.; Klimo, K.; Heiss, E.; Neumann, I.; Gamal-Eldeen, A.; Knauft, J.; Liu, G. Y.; Sitthimonchai, S.; Frank, N. *Mutat. Res.* **2003**, *523*−*524*, 163–172.

NP100078S